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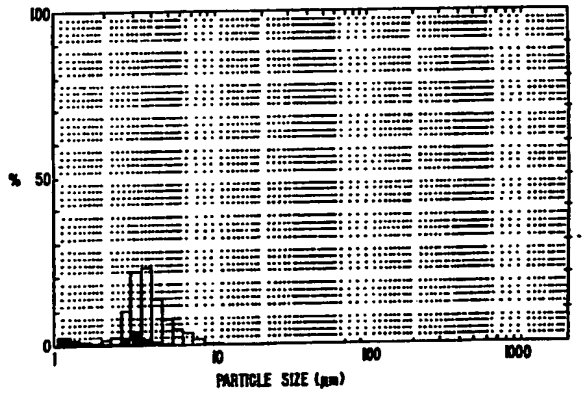
(54) Title: PREPARATION AND USE OF SOLID BEADS HAVING CONTROLLED CHARACTERISTICS

(57) Abstract

Processes for preparing and using controlled characteristic beads are disclosed, the process of making the beads including the steps combining at least one monoethylenically unsaturated monomer with a polyethylenically unsaturated monomer and a monomer-soluble initiator to form a monomer phase; combining the monomer phase with a liquid in which the monomer phase is substantially immiscible to form a reaction mixture such that when the monomer phase is vinyl in nature the liquid also includes a high molecular weight, high charge density surfactant, and when the monomer phase is acrylic in nature the liquid includes an organic polymer colloid suspending agent; agitating the reaction mixture at an initial speed and temperature for a time sufficient to homogenize the reaction mixture, thereby producing first intermediate droplets, and subsequently reducing the agitation speed to a level sufficient to allow limited coalescence of the first intermediate droplets to form second intermediate droplets having size distribution with less than about 30 % variance from the mean; and polymerizing the second intermediate droplets to form solid, substantially non-porous beads. One method of using controlled characteristic beads includes combining the beads produced by the process with a sample containing two separable components and centrifuging the sample for a time and at a rate sufficient to produce distinct bands of components.



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PREPARATION AND USE OF SOLID BEADS HAVING CONTROLLED CHARACTERISTICS

BACKGROUND OF THE INVENTION

This invention relates generally to methods of preparing and using solid beads having controlled characteristics, and specifically to preparing solid, non-porous beads having narrow size distribution which can be used for a variety of purposes including enhancing centrifugal separation of components in a sample by density differences, and to use of the beads to opacify or color plastic compositions.

Processes exist for the synthesis of beads having narrow particle size distribution, narrow size distribution being defined as a distribution having a standard deviation less than 30% of the mean. Classical suspension polymerization (stabilized oil in water suspensions utilizing oil soluble initiators, where a stabilizer or suspending agent acts to prevent particle coalescence as they proceed from liquid to solid states via a sticky phase) results in a large particle size distribution from which narrow size distribution materials can be obtained by the wasteful process of sieving. Quiescent suspension polymerization, as disclosed in U.S. Patent No. 2,932,629 (Wiley), has been used to generate particles greater than 180 μm with narrow particle size distribution by using an inorganic colloid stabilizer and "limited coalescence" (high agitation followed by a quiescent period). However, the processes outlined by Wiley are not amenable to the production of smaller particles. Classical emulsion polymerization (using surfactants to produce micelles into which hydrophobic monomers dissolve in aqueous medium) has been known to give particles

with a diameter of 0.1 to 0.2 μm and with a very narrow size distribution when special high charge density, high molecular weight surfactants are used, but a multitude of seeding steps are needed in order to bring the emulsion particles up to larger sizes. Crosses between emulsion and suspension polymerization have been patented, where both a conventional low molecular weight ionic surfactant (such as sodium alkyl sulfates) and oil droplet stabilizers (such as animal proteins) are used. Patterson discloses such a process in U.S. Patents 4,283,143 and 4,291,980. The beads produced have narrow size distribution, range in size from 0.2 to 1.0 μm, and are suitable for use as turbidity standards. Unfortunately, the beads must be subjected to steam distillation to hydrolyze the protein stabilizer to amino acids so that they may be removed by ion exchange resins.

It would be advantageous if an improved process for producing narrow size distribution particles having size ranging from about 2 to about 200 μm, more preferably from about 2 to about 40 μm, could be devised to produce the desired particles without the need for sieving or seeding, and without requiring extensive cleansing.

The uses of beads in hematology as centrifuge controls and as centrifugal separation aids are known. Lymphocytes can be separated from other formed elements of the blood by velocity sedimentation or by density-gradient centrifugation. Velocity sedimentation uses gradients of proteins such as albumin, dextran, or fetal calf serum, and the separation is a function of the radius of the cellular elements. Whether the separation is carried out by gravity or under the influence of centrifugation, the end result is an enrichment of the upper layers with the mononuclear cells, especially T and B cells. The second method, density gradient centrifugation, depends on the specific gravity of a gradient (either linear or discontinuous), made up by solutions such as albumin or Ficoll, a sucrose polymer with a molecular weight of about 400,000, available from Pharmacia AB, Uppsala, Sweden. It is highly soluble in water, and aqueous solutions have relatively low viscosity, owing to the spherical shape of the

ficoll molecule. The lighter cells (thos with the greatest nucleus/cytoplasm ratio) band nearest the top of the gradient, with each cell population seeking that specific gravity that corresponds to its own in the gradient. Separated lymphoid cells are collected from the interface by means of a Pasteur pipette and washed. Typically, to ensure good visibility, at least 2% of serum or a protein (e.g., bovine serum albumin) is included in the washing medium.

In this conventional centrifugation method, the shape and dimensions of the tube used to form the gradient do not affect the results of cell separation. Therefore, if the conditions of separation are adhered to rigorously, recovery of nearly 90% of the cells applied to the gradient can be anticipated. However, losses of as many as 30% of the cells may occur, usually owing to poor initial recovery at the interface or to improper washing procedures. Yields of less than 60% are unacceptable and may lead to distortions in the T and B cell ratios of the final preparation.

To improve lymphocyte recovery, centrifugation and cell sedimentation standards have been used in clinical settings to aid in the separation of cells based on density differences and also for the calibration of the instruments used for this purpose. Silicone oligomers have been used to aid in centrifugal separations but the relatively high viscosities of these materials can require higher rpm's and longer times for proper separation to occur. Polymer chips and beads have been used as density standards in the past, but a means of producing well defined materials for this purpose has not been available. Other prior art methods, by adding red cell rouleaux-enhancing agents such as hydroxyethyl starch, dextran, or modified gelatin to the whole blood, the sedimentation rate of the red blood cells is increased, and the granuloytes can be readily separated. It would be advantageous if methods of preparing and using narrow size distribution beads could be developed which avoid the shortcomings of prior art beads and their use in hematology and other fields.

Narrow particle size distribution beads are also useful as opacifiers for various uniaxially and biaxially

oriented thermoplastic films. U.S. Patents 4,377,616 and 4,632,869 disclose the use of beads made from nylons, polyamides, polyesters, polyethylene terephthalate acetals, acrylic resins, and polybutylene terephthalate. The beads must be incompatible with film matrix material in the sense that the beads form a distinct phase and act to cause voids to form when the extruded matrix is biaxially oriented, thus causing the film to have a lustrous, satin (opaque) appearance. Preferably the beads are spherical and range in size from 0.1 to about 10 μm. Frequently the beads do not produce adequately shaped or uniform size voids upon film orientation and inorganic fillers must be added to enhance the opacity, a cost disadvantage. Further, the beads do not have narrow size distribution, so that even a fraction of the total amount of beads used having size greater than the thickness of the extruded and oriented sheet or film may cause the film to rupture or bulge upon orientation.

It would be advantageous if beads having narrow size distribution and other controlled characteristics could be used to create voids of predictable size and shape so that more efficient light scattering by the voids would create opaque films more efficiently than presently used beads.

SUMMARY OF THE INVENTION

In accordance with the present invention a method of preparing solid beads having controlled characteristics, particularly having narrow size and/or density distribution, comprises combining at least one monoethylenically unsaturated monomer combined with a polyethylenically unsaturated monomer and an initiator to form a monomer phase. The monomer phase is then combined with an aqueous liquid phase which is substantially immiscible with the monomer phase to form a reaction mixture. If the monomer phase is vinyl in nature, a high molecular weight, high charge density surfactant is included in the aqueous phase, while if the monomer phase is acrylic in nature an organic polymer colloid suspending agent is included in the aqueous phase. The reaction mixture is then agitated at a speed and for a time sufficient to homogenize the

reaction mixture to form monomer droplets having diameter on th order of 1 μm (as checked with either a photoscope in th reactor vessel or by taking an aliquot and viewing under a microscope), at which point the agitation is reduced to allow limited coalescence of the droplets to form droplets having the desired diameter, as more fully explained herein. The droplets are then polymerized by conventional methods (e.g. heat, light) to form the desired controlled characteristic beads.

The process of making the beads as claimed herein produces beads with particle sizes, size distribution and density which are advantageous in light of previous processes since specific size, size distribution, and density beads can be easily tailored to the end use of the beads. Previous processes either produce too small beads which require seeding to produce larger beads, or too large beads requiring sieving, both disadvantages being overcome by the present invention.

Thus, another aspect of the present invention is use of acrylic beads having both narrow size and density distribution as separation aids and calibration controls in centrifugal separation of components in a sample, such as in conventional density gradient centrifugal separation of whole blood into its various cellular components. A preferred bead composition for this use comprises the reaction product of copolymerizing n-butylmethacrylate with ethylene glycol dimethacrylate (EGDMA). When used as separation aids, isolation of components in a material can be facilitated by the density specific materials as their density can be used to enable them to localize at an interface, thus facilitating visualization of the interface, or by collecting in component bands having the same density, thereby enhancing visibility of the components. Depending on the quantity of beads used, the density specific beads function as an aid in the collection of the various separated components by preventing mixing of the components at their interface.

The density specific acrylic beads may also be used as calibration standards for centrifuges used in hematology, blood banking, and blood plasma collection apparatus, as well as in other instances where centrifuges must be calibrated.

Due to the size, density, and size distribution of the beads, they behave as very well-defined cells for calibration purposes. Further, the ability to color the beads or have them selectively adsorb dye from the solution greatly increases their visibility and thus their usefulness for detection, even by the unaided eye.

Another aspect of the present invention is use of narrow size distribution beads formed from vinyl monomer phases described herein as fillers and opacity enhancers in uniaxially and biaxially oriented thermoplastic films such as polypropylene. One preferred bead composition for this use comprises the reaction product of copolymerizing styrene with divinylbenzene. The ability to make substantially uniform, spherical, solid, non-porous beads with controlled particle sizes enhances the efficiency of opacification in processes as described in U.S. Patents 4,377,616 and 4,632,869 since both substantially uniform size and shape voids are produced with the benefit of more consistent opacity of polypropylene or other plastic film or sheet due to the more predictable light scattering produced by the voids.

The amount and type of monomer, surfactant or colloid, degree of agitation, temperature, etc. of the process of making the beads can be manipulated to produce beads with different characteristics depending on the use of the beads. For example, solid, non-porous acrylic beads with a narrow weight average size distribution (preferably less than 25% variance), with particle sizes ranging from about 0.1 to about 200 μm can be produced for hematology uses, as described hereinbelow, using acrylic monomers and polyvinylalcohol as colloid suspending agent. The preferred bead size for this application ranges from about 2 to about 20 μm, more preferably from about 6 to about 9 μm, the latter about the size of red blood cells. The specific gravity of the beads in hematology service ranges from about 1.0 to about 1.1, more preferably from about 1.05 to about 1.08, the actual specific gravity depending on the particular blood components to be separated. In thermoplastic film opacification, the beads are vinyl in nature and are preferably spherical and also of narrow size

distribution, with sizes ranging from about 0.1 to about 10 μm, more preferably ranging from about 0.1 to about 5 μm. In this service the bead density is not critical, with specific gravity ranging from about 1.03 to about 1.05, which may be tailored by substitution of a percentage of another (lighter or heavier) vinyl monomer for styrene, such as vinylxylene.

Further aspects of the invention will become apparent with reference to the detailed description which follows herein.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 shows histograms from a particle sizing machine as used in example 4.

DESCRIPTION OF PREFERRED EMBODIMENTS

The method of forming the solid, non-porous controlled characteristic polymer beads in accordance with the present invention, includes combining at least one monoethylenically unsaturated monomer with a polyethylenically unsaturated monomer in the presence of a monomer soluble free radical initiator or catalyst to form a monomer phase. The density of the final beads is controlled largely by the type and proportion of the monomers. This phase is then combined (either before or at the time of entering the homogenization vessel, explained below) with a liquid in which the monomer phase is substantially immiscible to form a reaction mixture. The immiscible liquid includes a high molecular weight, high charge density surfactant in the case where the monomer phase is vinyl in nature and an organic polymer colloid suspending agent when the monomer phase is acrylic in nature. The reaction mixture is then agitated at a speed and for a time sufficient to homogenize the reaction mixture and produce first intermediate droplets. As this step entails a high degree of shearing action, a large amount of heat is evolved and temperature must be monitored and controlled to prevent premature polymerization at the homogenizing conditions, the maximum temperature depending on the activity of the initiator (the higher th activity, the lower the temperature must b).

The size and size distribution is checked with a photoscope in the reactor or a sample is taken to an off-site microscope for examination. Additional surfactant or suspending agent may then be added and/or the homogenization conditions changed to ensure substantially complete homogenization, i.e., no significantly large clumps of droplets are found. Water may also be added to reduce solution viscosity. The agitation is then slowed to allow limited coalescence of the first intermediate droplets to form second, larger intermediate droplets, and polymerization initiated to form the controlled characteristic beads. The bead preparation processes described herein result in the formulation of spherical beads, but beads having non-spherical asymmetric, and/or irregular geometries will also find use so long as they meet the necessary physical parameters set forth below.

It is well known that oxygen acts as an inhibitor of free radical polymerization and should, therefore, be excluded. The preferred embodiments of this invention effect polymerization under substantially anaerobic conditions.

Suitable catalyst which provide free radicals which function as reaction initiators include benzoyl and lauryl peroxide.

The amount of peroxide catalyst required is roughly proportional to the concentration of the mixture of monomers. The usual range is 0.01% to 3% of catalyst with reference to the weight of the monomer mixture. The preferred range is from 0.2% to 1.5%. The optimum amount of catalyst is determined in large part by the nature of the particular monomer selected, including the nature of the impurities which may accompany said monomers. Other suitable classes of free radical generating compounds are known to those skilled in the art, as well as other methods of effecting copolymerization of the compositions of the present invention, for example, subjecting the reaction mixtures to ultraviolet radiation in the presence of suitable catalyst at ambient or slightly elevated temperatures.

Preferred methods of making the controlled characteristic polymeric bead are novel combinations of emulsi n and susp nsion polymerization t chniques in an

ana robic (nitrogen blanketed) liquid-liquid system. A monomer solution containing monomers and a polymerization initiator or catalyst is form d.

Preferred monoethylenically unsaturated monomers generally suitable for preparing polymer beads for hematology service are acrylic in nature and include esters of acrylic and methacrylic acid, including the methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, amyl, hexyl, octyl, ethylhexyl, decyl, dodecyl, cyclohexyl, isobornyl, phenyl, benzyl, alklphenyl, ethoxymethyl, ethoxyethyl, ethoxypropyl, propoxymethyl, propoxyethyl, propoxypropyl, ethoxyphenyl, ethoxybenzyl, and ethoxycyclohexyl esters; 2-(dimethylamino) ethyl methacrylate; vinyl esters, including vinyl acetate, vinyl propionate, vinyl butyrate and vinyl laurate; and the like.

Exemplary monoethylenically unsaturated monomers generally suitable for preparing polymer beads for opacification service are vinyl in nature and include styrene, vinyltoluene, vinylxylene, vinyl chloride, vinylidene chloride, vinyl acetate, N-vinyl pyrrolidone, 2-vinyl pyridine, and vinyl alkyl ethers with alkyl ranging from C_2 - C_8, ethylvinylbenzene, dicyclopentadiene; vinyl esters, including vinyl acetate, vinyl propionate, vinyl butyrate and vinyl laurate; vinyl ketones, including vinyl methyl ketone, vinyl ethyl ketone, vinyl isopropyl ketone, and methyl isopropenyl ketone; vinyl ethers, including vinyl methyl ether, vinyl ethyl ether, vinyl propyl ether, and vinyl isobutyl ether; vinyl pyridine; and the like.

Preferred polyethylenically unsaturated monomers suitable for forming vinyl beads are selected from the group consisting of divinylbenzene, divinyltoluene, and divinylxylene, divinylketone, divinylsulfide; alkyldivinylbenzenes having from 1 to 4 alkyl groups of 1 to 2 carbon atoms substituted on the benzene nucleus; alkyltrivinylbenzenes having 1 to 3 alkyl groups of 1 to 2 carbon atoms substituted on the benzene nucleus; trivinylnaphthalenes, and polyvinylanthracenes.

Exemplary polyethylenically unsaturated cross-linking monom rs suitable for preparing acrylic b ads for hematology and other centrifugation processes include diallyl phthalate, ethylene glycol diacrylate, ethylene glycol dimethacrylate, trimethylolpropanetrimethacrylate, divinylsulfone; polyallyl ethers of ethylene glycol, of glycerol, of pentaerythritol, of diethyleneglycol, of monothio-and dithio- derivatives of glycols, and of resorcinol; allyl acrylate, diallyl maleate, diallyl fumarate, diallyl succinate, diallyl carbonate, diallyl malonate, diallyl oxalate, diallyl adipate, diallyl sebacate, divinyl sebacate, diallyl tartrate, diallyl silicate, triallyl tricarboxlylate, triallyl aconitate, triallyl citrate, triallyl phosphate,

Monomers may be introduced to provide beads which adsorb dyes out of solutions or modified (for example by surface charges such as produced by SO_3^- or CO_2^- when dyes having positive charges are used) to have groups which adsorb dyes.

A particularly preferred monomer system for use in hematology service of the present invention is formed by the copolymerization of n-butylmethacrylate and ethylene glycol dimethylmethacrylate (EGDMA). Usually, the n-butylmethacrylate will be present at from about 50 to 90 percent of the monomer mixture, more usually at about 60 to 80 percent of the monomer mixture, typically being in the range from about 65 to 75 percent of the monomer mixture, with the ethylene glycol dimethylmethacrylate, initiator, and possibly a heat transfer diluent such as toluene forming the remainder of the mixture. As the amount of n-butylmethacrylate increases, the percentage of crosslinking (determined as percentage of glycol diacrylate monomer to total of alkyl acrylate monomer and glycol diacrylate monomer) decreases.

Another particularly preferred monomer system for controlled characteristic bead formulations is formed by copolymerization of styrene and divinylbenzene, which are useful in both centrifugation and opacification. Usually, the styrene will be present at about 40 to 90 percent of the monomer mixture, more usually being present at about 45 t 80

percent, with divinylbenzene and possibly a density adjustment portion of a third monomer such as vinyltoluene forming the remainder of the mixture.

After combining the appropriate monomers, the monomer phase is then preferably combined with an aqueous solution, which generally contains additives such as high molecular weight, high charge density surfactants when narrow size distribution beads are desired and dispersants (suspending agents) when density specific beads are desired, to promote the suspension.

Preferable surfactants include a wide range of substances, notably inert, disulfonated organics. Particularly preferred examples of such surfactants are alkylated, disulfonated diphenyloxides, the hydrophobe having from 5 to 16 carbon atoms (straight or branched chain) and will be liquid substances which meet the criteria listed in Table 1. Commercially available surfactants meeting the criteria of Table 1 include DOWFAX™ anionic surfactants from Dow Chemical Company. Especially preferred is DOWFAX™ 2A1, which has as hydrophobe branched C_{12}; an active content of 45%; average molecular weight of 569; and weight percent for critical micelle concentration (cmc) of 0.4. Mixed surfactants having different cmc, molecular weight, etc., will frequently find use in providing beads having differing size and size distribution.

Preferred suspending agents useful in producing acrylic beads should prevent the monomer droplets from coalescing or sticking together while in their non-solidified form, and be easily removable from the solidified beads. An important consideration is the aqueous/oil ratio (A:O). For best results in terms of efficiency in classical suspension and emulsion polymerization the A:O ratio is as low as possible (the lower limit due ultimately to viscosity and heat transfer requirements). Thus volumetric efficiency increases as A:O decreases. Particularly preferred suspending agents are exemplified by water soluble organic polymer polyols which can function at A:O ratios ranging from about 1 to about 5. These include polyvinylpolyols having molecular weight of at least 40,000, more pref rably having a molecular weight of at least

50,000. Polyvinylalcohol itself cannot be made directly but can be made by first polymerizing vinyl ac tate and then hydrolyzing the polymer to the polyol. Hydrolysis of the acetate can be carried out in concentrated methanol solution with a sodium methoxide catalyst. Various degrees of hydrolysis are marketed, with the percentage hydrolyzed for the present invention ranging from about 50 to about 90%, more preferably ranging from about 70 to about 90%.

Table 1
Preferred Surfactant Properties*

Property	Broad Range	Preferred Range
Hydrophobe	C_6 - C_{16}	C_{10} - C_{14}
Active Content (%)	30 - 50	40 - 50
Avg. Mol. Wt.	450 -650	550 - 600
Surface Tension in Deionized Water (dynes/cm):		
0.1% Active Solution	20 - 50	25 - 35
1.0% Active Solution	20 - 50	25 - 35
Interfacial Tension of 0.1% Surfactant vs. kerosene (dynes/cm)	1.0 - 20	1.0 - 5.0
Hydrophile/lipophile Balance (HLB)	10 - 30	10 - 20
Weight % for Critical Micelle Conc. (By Conductivity)	0.05 - 5.0	0.1 - 1.0

* Adapted from Dow Chemical Company Tech. Serv. Bull. #192-1011-86

Once the monomer solution is combined with the aqueous medium, the mixture is agitated vigorously for a time and speed sufficient to homogenize the monomer/aqueous solution to form discrete first intermediate droplets. The homogenized mixture is then viewed either by a photoscope attached to the homogenization vessel or samples of the solution taken and the size and size distribution of the first intermediate droplets determined by methods well known in the art. More surfactant or suspending agent, depending on the monomer system, may be added at this point, to break up large clumps of monomer which

are apparent upon viewing. The agitation required for a particular batch or continuous flow process may be easily determined, depending on the amount of monomer to be polymerized. Homogenizing conditions are then carried on for a time over and above that usually known to be necessary for that particular amount of monomer. As mentioned above, since a great amount of mechanical energy is introduced into the system as shear, the homogenization vessel must be held to a maximum temperature, which is dependent on the activity of the free radical initiator used. If benzoyl peroxide is used as initiator, the temperature should preferably be kept below about 50°C, although some degree of polymerization while homogenizing is acceptable (a degree of polymerization of about 20% being considered unacceptable). If a more reactive initiator is used, such as 2,2'-Azobisisobutyronitrile (AIBN), the temperature should be kept below about 40°C, and so on.

Homogenization of the reaction mixture is a critical aspect of the method of producing the solid, non-porous beads of the present invention. The homogenizing conditions may be produced by handheld devices or by specially designed prepackaged homogenization systems, or may simply be achieved by a flow restriction in a pipeline. The homogenizing conditions used in producing the solid, non-porous beads having narrow particle size allow the use of high molecular weight surfactants and suspending agents in the polymerization process, which in combination with the high degree of agitation, form beads having the narrow particle size distribution and sizes desired in the products discussed herein. Homogenizers used in creating the homogenizing conditions herein should be capable of speeds of up to at least 20,000 rpm, with the capability of being turned down to about 200 rpm to allow limited coalescence. Homogenizers are commercially available from the following manufacturers: IKA-Works, Cincinnati, Ohio (rotor-stator design) and Arde-Barinco (impeller type). The rotor-stator design is preferred in that it is somewhat more efficient in producing the homogenizating conditions as it produces droplets having narrower size distribution than the impeller types, and has a higher capacity

rating. The rot r-stator design also allows easy scale up of th process.

Agitation is then reduc d to allow limited coalescence of the beads to the desired second intermediate droplet size, and polymerization is effected (typically by activating the reactants by either increased temperature or irradiation). Once polymerization is complete, the resulting beads are recovered by filtration and washed with acetone and deionized water. The beads at this point are solid, substantially non-porous structures, the polymer having formed in a micelle (if a surfactant is used) or suspended monomer droplet (if suspending agent is used). (The terms "first intermediate droplet" and "second intermediate droplet" are meant to include micelles formed when using the surfactants.)

The beads produced by the novel methods described herein may be recovered by filtration, preferably using vacuum apparatus (such as a Buchner funnel). To obtain "clean" beads, for example where impurities might interfere in downstream processing, the beads are then washed with an appropriate solvent to remove organic species not bound to the polymer, including surfactants and suspending agents having deposited on the bead surfaces from the aqueous phase, unreacted monomers and residual initiators and catalysts. Examples of such solvents include isopropanol and acetone, either alone or in aqueous solution. Once washing is complete, the solvent itself is removed by drying, for example in a vacuum. Alternatively, in some uses retention of surfactant and/or suspending agent on the bead surface is beneficial, for example, in centrifugation service, where the beads are typically resuspended from the powder form. In centrifugal separation service, since the initially powdered beads are typically resuspended in aqueous solutions with a suspending agent anyway prior to use, a quite substantial amount of the organic polymer colloid suspending agent can remain on the beads' surface. Surfactants employed will ordinarily have to be substantially removed (although to no critical degree) from the beads prior to their use as opacifiers as they may form undesirable carbonaceous char at extrusion temp ratures. Removal of the DOWFAX™ surfactant(s),

if desired, may be effected by calcium salt coagulation and subsequent water washing.

In certain cases, an alternative method of cleaning may be used - i.e., where the surfactant/suspending agent, unreacted monomer, and water will form an azeotrope. In these cases, steam distillation is an effective way of cleaning the beads. This again may be followed by drying under vacuum.

The polymerization process used in preparing the controlled characteristic beads of the present invention is advantageous in that it can be modified to control the size, size distribution, and density of the beads. Bead size and size distribution are controlled primarily by the degree of agitation and the amount and properties of surfactant, with more rigorous agitation and lower cmc causing smaller droplets and hence smaller polymerized beads. The bead density, in contrast, is controlled by degree of agitation, the cross-linking density, suspending agent amount and properties, and monomer system used. Density is generally increased by changing the amount of polyethylenically unsaturated monomer used, or by increasing the concentration of a denser monoethylenically unsaturated monomer in the monomer mixture, or both. An increase in denser monomer tends to increase bead density and hence the settling velocity of the beads. Bead diameter is also affected by the concentration of suspending agent in the immiscible phase, if used. In density gradient centrifugation, as explained further herein, the beads are all preferably the same size even though they may have different densities.

A general discussion of separation of components of liquid samples by taking advantage of density differences between particles suspended in the sample is discussed in Kirk-Othmer, *Encyclopedia of Chemical Technology*, 3rd Ed., John Wiley & Sons, Vol. 4, pp.194-218. This discussion summarizes general principles of centrifugal separation as it relates to the size and density of components to be separated. A single solid particle or discrete liquid drop settling under the acceleration of gravity in a continuous liquid phase accelerates until a constant terminal velocity is reached. At

this point the force resulting from gravitational acceleration and the opposing force resulting from frictional drag of the surrounding medium are equal in magnitude. The terminal velocity largely determines what is commonly known as the settling velocity of the particle or drop under free-fall or unhindered conditions; for a small spherical particle, it is given by Stokes' Law.

The assumptions and conditions set forth as a basis for deriving the mathematical equations describing the sedimentation of particles in centrifugal fields are as follows: concerning the particulate material, the particles (or drops) are spherically shaped and uniform in size, and they should not deaggregate, deflocculate, coalesce, or flocculate during their passage through the zone in which separation occurs; concerning the flow conditions, the major assumption is that remixing at the interface of the separated materials is negligible.

Few of these assumptions are fully satisfied in practice. Potential interference between the separated phases can occur and may lead to apparently poor sedimentation performance if an excessive volume of the sedimented phase is retained in the centrifuge. As explained in the Background of the Invention, in hematology service poor cell separation at the interface is one main cause of poor or unacceptable cell recovery. The controlled characteristic beads made by the processes of the present invention allow easier visualization of the interface for economical recovery of cells.

In using centrifugation (sedimentation) techniques in blood fractionation, these techniques take advantage of the differences in density between blood plasma and suspended cells and differences in size and density among the cell types. As noted in Table 2, such methods separate whole blood into four fractions: plasma, platelet concentrate, white cell (leukocyte) concentrate, and leukocyte-poor packed red blood cells.

The use of controlled characteristic beads as produced by the methods described herein is especially useful in the isolation of l ukocyte concentrates and leukocyte-poor

packed red blood cells. Visual inspection of the packed cell mass remaining after centrifugation of a unit of whole human blood and removal of the platelet-rich plasma will normally reveal the presence of a white "buffy-coat" consisting of white blood cells (WBC, leukocytes) above the packed red blood cells (RBC). Red blood cells tend to sediment faster than leukocytes, owing to their higher density (Table 2) and an ability to form rouleaux (an arrangement analogous to a roll of coins). However, only a partial separation of red blood cells from white blood cells or of white cell types from each other can be obtained by centrifugation. Other prior art methods, by adding red cell rouleaux-enhancing agents such as hydroxyethyl starch, dextran, or modified gelatin to the whole blood, the sedimentation rate of the red blood cells is increased, and the granulocytes can be readily separated. Problems occur in bottle centrifuges however which operate discontinuously and where the interface may degrade upon stopping the machine.

Table 2
Specific Gravity and Diameter of Blood Components*

Component	Specific gravity	Diameter, μm
red blood cells (erythrocytes)	1.093-1.096	7.2-7.9
white blood cells:		
granulocytes	1.087-1.092	10-14
monocytes	1.075-1.080	15-22
lymphocytes	1.070	10-20
platelets	1.040	1-2
plasma	1.025-1.029	--------

* From Kirk-Othmer, *Encyclopedia of Chemical Technology*, 3rd Ed., John Wiley & Sons, Vol. 4, pp. 25-36.

As explained in the Background Of The Invention, a primary emphasis by many laboratories at present in leukocyte fractionation research is the development of techniques for the selective isolation of specific leukocyte cell types. The most popular method for the preliminary fractionation of leukocyte cell types is that of Boyum, which uses Ficoll-Hypaque discontinuous density gradients to separate mononuclear cell types from granulocytes. In this research it is very important that the "buffy-coat" from a unit of centrifuged whole blood be

easily obtained as it is an excellent starting material for these procedures. The beads and methods of using of the present invention, when formulated with the pref rred bead size and size distribution (i.e., 6-9 microns, the approximate diameter of red blood cells and less than about 30% variance in size distribution) may be used to enhance the separation and visualization of the separation of the "buffy-coat" from various other blood components discussed herein.

Thus, another aspect of the present invention is a method of using density-specific, narrow size distribution beads as a separation aid in centrifugation comprising combining a solution containing at least one narrow density range of beads with a sample having at least two components to be separated to form a test sample, and centrifuging the resulting test sample for a time and rate sufficient to separate the components in the test sample into at least two substantially distinct bands of components, the beads thereby functioning to enhance visualization of the separated component bands. One preferred application of this general method is in the immediately preceding discussion of blood fractionation where the density-specific beads are formulated in three specific gravity ranges: a first ranging from about 1.04 to about 1.05, the second ranging from about 1.05 to about 1.06, and the third ranging from about 1.06 to about 1.08. More preferably, the first specific gravity standard range has specific gravity ranging from about 1.043 to about 1.047, the specific gravity of the second standard ranges from about 1.054 to about 1.058, and the specific gravity of the third standard range is from about 1.065 to about 1.075. It can be seen from viewing Table 2 that these specific gravity ranges approximate the specific gravi- y of blood platelets, lymphocytes and monocytes, and granulocytes, respectively.

The solution containing at least one narrow density range of beads includes beads made by the process above described, the density ranges controlled by controlling the percentages of specific monoethylenically unsaturated monomers used. For example, substantially pure n-butyl methacrylate will produce a polymer having density of about 1.055 gm/cc at

20°C, while t-butyl methacrylat will produce a polymer having density of about 1.022 gm/cc at 20°C, and so on. See generally Kirk-Othmer, *Encyclopedia of Chemical T chnology*, 3rd Ed., John Wiley & Sons, Vol. 15, p. 379. Thus mixtures of these monoethylenically unsaturated monomers would produce a copolymer with EGDMA having density somewhere between the two extremes.

The centrifugation step of the method entails centrifuging for a time and rate sufficient to separate the components in the test sample into at least two substantially distinct bands of components, the beads thereby functioning to enhance visualization of the separated component bands. As used herein this phrase means that the operator of the centrifuge spins the container holding the sample and beads long enough for a degree of component separation to take place that may be easily visualized by the operator, another person, or an automated device operating on the same principle of visual detection as a human. The term "substantially distinct bands" of course depends somewhat on the person or device viewing the sample, but the beads as made by the processes described herein will typically produce shorter spin times, all else being equal, for the "distinct bands" to be visualized. The "at least one density range of beads" can either comprise a substantially single density, where the beads aggregate between two bands of components, or more than one distinct density range, where the beads either compose part of two or more bands or separate two or more bands.

The method of using beads as a centrifugation separation aid may be further optimized by using beads having surface modifications with a moiety selected from the group consisting of SO_3^- and CO_2^- to facilitate adsorption of dyes. Such dyes include Acridine Orange; Cibicet Orange, 2R; Supracet Fast Brown, 5R; etc.

The particularly preferred bead material for centrifugation service is a copolymer produced by the process described above of n-butylmethacrylate crosslinked with ethylene glycol dimethacrylate (EGDMA). Another particularly pr ferred bead material is n-hexylmethacrylate, also cross-

linked with EGDMA. Tripolymers of two monoethylenically unsaturated monomers and EGDMA or another polyethylenically unsaturated monomer can be used to control bead density, as previously discussed.

The controlled characteristic beads used in this service preferably have sizes ranging from about 2 to about 20 microns, more preferably ranging from about 6 to about 9 microns, with a bead size distribution (weight average) variance of less than about 30%, more preferably less than about 25%.

A closely related use of the controlled characteristic beads produced by the methods described herein relates to a method of using density-specific, narrow size distribution beads to calibrate a hematology centrifuge comprising combining at least two narrow specific gravity ranges of beads with blood to form a control, the blood having substantially all platelets, lymphocytes/monocytes, and granulocytes removed therefrom, the beads in each specific gravity range having a size distribution variance of at most 30%, and centrifuging the control to determine if the centrifuge is calibrated. A preferred method of calibrating a hematology centrifuge comprises using three standards having specific gravity ranging from about 1.04 to about to about 1.05, the specific gravity of the second standard ranging from about 1.05 to about 1.06, and a specific gravity of the third standard ranging from about 1.06 to about 1.08. A more preferred method uses a first specific gravity standard ranging from about 1.043 to about 1.047, specific gravity of the second standard ranging from about 1.054 to about 1.058, and specific gravity of the third standard ranging from about 1.065 to about 1.075. This method of course can be easily extended to centrifugal separation of other samples as will be apparent to those skilled in the art.

One particularly preferred bead material for centrifugation standards is n-butylmethacrylate cross-linked with EGDMA, while another is n-hexylmethacrylate cross-linked with EGDMA, with density varied as previously discussed.

The bead size and variance in size distribution for the method of calibrating a centrifuge in hematology service are similar to the method of using the beads as centrifugal separation enhancers. Preferred bead size ranges from about 2 microns to about 20 microns, more preferably ranging from about 6 microns to about 9 microns, with a size distribution preferably less than about 25%. The beads may also be surface modified in the hematology control with moiety selected from the group consisting of SO_3^- and CO_2^- to facilitate adsorption of dyes, which of course would be done before the beads are put in a container to form the control (i.e., pre-dyed beads).

Another aspect of the invention involves a kit for calibrating centrifuges, which kit comprises a sealed container having disposed therein blood having substantially all platelets, lymphocytes and monocytes, and granulocytes removed therefrom, and replaced respectively with three sets of solid, non-porous beads, the first set having specific gravity approximating that of platelets, the second set having specific gravity approximating that of lymphocytes and monocytes, and the third set having specific gravity approximating that of granulocytes. All beads in each of the three specific gravity ranges have size ranging from about 2 to about 20 microns, preferably from about 6 to about 9 microns, with less than about 30% variance in size distribution, preferably no more than about 25% variance. The chemical composition of the beads is that described hereinabove in reference to the method of using controlled characteristic beads in hematology service. A preferred form of this kit includes kits where one or more sets of beads is surface modified with chemical moieties selected from the group consisting of the group of SO_3^- and CO_2^-, the moieties in turn coupled to dyes (i.e., pre-dyed beads).

Another kit for hematology services includes a kit for enhancing visibility of centrifugal separation between one or more components of a sample, the kit comprising at least tw different density bead groups contained in the same or different container, the beads being substantially solid and non-porous and having size ranging from about 2 to about 20 microns, preferably ranging from about 6 to about 9 microns,

with particle size distribution variance less than about 25%. The preferred chemical composition of the beads is that described hereinabove in reference to the method of using controlled characteristic beads in hematology service. As with the kits previously described for calibrating centrifuges, the beads of one or more groups may be surface modified with moieties to adsorb dyes from a sample. A particularly preferred kit for enhancing visibility of centrifugal separation includes a kit wherein the sample is human whole blood and at least two different density groups comprise a first set having a specific gravity of about 1.04 to about 1.05, more preferably from about 1.043 to about 1.047, the second group having specific gravity ranging from about 1.05 to about 1.06, preferably from about 1.054 to about 1.058, and the third specific gravity standard ranging from about 1.06 to about 1.08, preferably from about 1.065 to about 1.075.

A typical commercially available centrifuge which may be used in accordance with the beads of the present invention include the GS-6 Series centrifuges available from Beckman Instruments, Inc., Palo Alto, California, which generates up to roughly 5700 g which can process up to three liters per run.

Another novel aspect of the present invention includes a method of using controlled characteristic beads produced by the processes described above as opacifiers for plastic compositions comprising providing a resin combination comprising a thermoplastic polymer matrix having dispersed therein as distinct phases a multiplicity of small spherical solid particles formed from monomers selected from the group consisting of vinyl monomer phases described above; forming an unoriented film of said resin combinations; and biaxially orienting said film to an extent sufficient to opacify the film. A preferable film material is polypropylene while the particularly preferred vinyl bead material is styrene/divinylbenzene copolymer. The techniques used for extruding polymer films having opacifying beads therein are described in U.S. Patents 4,377,616 and 4,632,869 may be similarly used in the process described herein, the disclosure of th s two patents incorporated by reference in their

entirety. For example, as described in U.S. Patent 4,362,869, a master batch technique is employed either in the case of forming the spherical particles *in situ* or in adding preformed spheres to a molten thermoplastic matrix material. After the formation of a master batch, appropriate dilution of the system can be made by adding additional thermoplastic matrix material until the desired proportions are obtained.

As stated in U.S. Patent 4,632,869, the beads can be present in up to about 20% by weight of the film total weight with a preferred range from about 2 to about 10% by weight. The beads made by the novel processes described herein preferably have very little surfactant or suspending agent remaining on their surface as the possibility exists that the surfactants and suspending agents would char at the surface temperatures of the extrusion (circa 250° C). It is preferred that the degree of opacity of the oriented film be less than 70% light transmission. Due to the uniformity of size and shape of the cavities formed by the beads of the present invention, it is not anticipated that pigments, such as titanium dioxide, color oxides and the like, would be required, however, the opacity of the film can be enhanced by the inclusion in the film of from about 1 to about 5% by weight of these pigments. Thermoplastic materials for the film can be any thermoplastic resin material which is incompatible with styrene-divinylbenzene beads and which can be oriented in film form. By incompatible, it is meant that distinct phases of the two materials will result when inter-blending of the two is attempted. Examples of such thermoplastic resins include the polyolefins, polyethylene, polypropylene, polybutylene, etc. Included are also the distinct species of these materials such as low density polyethylene (LDPE), ultra low density polyethylene (ULDPE), high density polyethylene (HDPE), etc.

The general method for forming opaque oriented film using the beads of the present invention is accomplished by a slot extruding a film of resin composition (matrix plus styrene-divinylbenzene) and thereafter sequentially biaxially orienting the film. During the orientation, a stratum of voids is formed in the matrix polymer. Since th styrene-

divinylbenzene beads are incompatible with the matrix material, during machine direction orientation, each sphere tends to create a streamlined void. During subsequent transverse orientation, the transverse dimension of this void is correspondingly increased. During these steps, the film turns a bright while pearlescent opaque color.

The preferred controlled characteristic beads used in the methods of opacifying plastic compositions comprise styrene-divinylbenzene beads which are spherical, non-porous, solid beads, having particle size ranging from about 0.1 to about 10 microns, more preferably ranging from about 0.1 to about 5 microns. The particularly preferred beads have size distribution variance less than about 30%, more preferably less than about 25%. Although not critical in opacification service, the beads generally have a specific gravity ranging from about 1.03 to about 1.05 when styrene divinylbenzene beads are used.

The beads made by the process of the present invention are substantially solid and non-porous, and preferably spherical in opacification service, while some degree of elasticity and non-sphericity is allowable centrifugation service. In both cases the beads produced and used are substantially spherical, and are preferably non-degradable. It is important when the beads are being made and in both centrifugation and opacification service that the beads do not stick together in solution, although agglomeration at an interface is helpful in centrifugation service. The beads used in opacity service may be somewhat porous due to crazing which occurs on stressing the films into which they are combined.

Suitable polymeric beads will not readily undergo unwanted reactions, will be stable over a wide pH range, and will resist moderate oxidation and reduction. The particles should be stable at higher temperatures (for opacification service, at least to 250 °C) and g forces (for centrifugation service) have a relatively long shelf life. Desirable physical parameters for the solid, non-porous beads are described in Table 3. The beads may swell or contract in various solvents to m et th size requirements of various medical and industrial

disciplines. For example, polybutylmethacrylat (poly(BuMA)) beads cross-linked with ethylene glycol dimethacrylate (EGDMA) swell in acetone and hexane and shrink in water.

The beads useful in centrifugation service in general, and hematology centrifugation specifically, may be formed from a wide variety of acrylic polymers such as polyalkylmethacrylates, polyvinyl acetate, and polyhydroxyalkyl methacrylates. Preferred beads include those made from at least one monoethylenically unsaturated monomer selected from the group consisting of acrylic acid, methacrylic acid, alkyl acrylates having alkyl group of C_1 - C_6, normal and branched alkyl methacrylates having alkyl group of C_1 - C_6, and acrylamide, and a polyethylenically unsaturated glycol diacrylate selected from the group consisting of

$$CH_2=C(R^1)C(O)O[(CH_2)_yCH(R^2)O]_zC(O)CH(R^1)C=CH_2$$

wherein R^1 is H or CH_3;
R^2 is H or CH_3;
y is 1 to 5; and
z is 1 to 14.

Beads useful in opacification service include beads made from monomer phases including at least one monoethylenically unsaturated monomer selected from the group consisting of styrene, vinyltoluene, vinylxylene, vinyl chloride, vinylidene chloride, vinyl acetate, N-vinyl pyrrolidone, 2-vinyl pyridine, and vinyl alkyl ethers with alkyl ranging from C_2 - C_8, and a polyethylenically unsaturated monomer is selected from the group consisting of divinylbenzene, divinyltoluene, divinylxylene, divinylketone, divinylsulfide; alkyldivinylbenzenes having from 1 to 4 alkyl groups of 1 to 2 carbon atoms substituted on the benzene nucleus; alkyltrivinylbenzenes having 1 to 3 alkyl groups of 1 to 2 carbon atoms substituted on the benzene nucleus; trivinylnaphthalenes, and polyvinylanthracenes.

The preferred polymeric beads of the present invention comprise solid, non-porous polymeric beads having properties as shown in Table 3. Such beads are mechanically stable compared with non-rigid materials, allowing manufacturing, processing, and handling of the beads under

relatively rigorous conditions which might result in the rupture or damage of less stable materials.

Table 3
Controlled Characteristics of Beads

Property	Broad Range	Preferred Range
Size (hematology)*	2 - 20 μm	6 - 9 μm
Size (opacification)*	0.1 - 10 μm	0.1 - 5 μm
Spec. Grav. (hematology)	1.00 - 1.10	1.05 - 1.08
Spec. Grav. (opacification)	1.03 - 1.05	----------
Size Distribution@	< 25 %	< 30 %

* weight average
@ variance from the mean, weight average

The solid polymeric beads useful in opacification will have greater than 10% cross-linking, usually having in the range from about 15% to 80% cross-linking, more usually having in the range from about 25% to 60% cross-linking. In the case of gel-like bead products, the cross-linking will be substantially less, usually being from about 0.1% to 5%, as in polyalkylmethacrylate beads for hematology controls. The calculated or theoretical percentage of cross-linking is defined as the weight of polyethylenically unsaturated monomer (or monomers) divided by the total weight of monomer, including both polyethylenically unsaturated and monoethylenically unsaturated monomers. The term "solid" as used herein is meant to mean not rigid over all temperature ranges, i.e., the beads become solid but non-rigid at temperatures above their glass transition temperature (T_g).

The production and use of controlled characteristic beads useful in the methods of the invention will be further illustrated in the examples that follow wherein, for example, the density is controlled by reaction of, for example, styrene and divinylbenzene, in which a known amount of the styrene monomer is substituted with a heavy or lighter monomer such as vinyltoluene. The styrene-divinylbenzene comonomer pair is an especially preferred comonomer pair in opacificati n service

because f the chemical stability of styrene-divinylbenzene copolymer. As would be apparent to one skilled in the art, the term "divinylbenz ne" as used in this description, as well as in the appended claims, is meant to include pure divinylbenzene, as well as commercial divinylbenzene, which is really a mixture of divinylbenzene and ethylvinylbenzene.

EXPERIMENTAL

EXAMPLES OF METHODS OF MAKING BEADS

Example 1: Butylmethacrylate Beads, 2% Cross-linking

1.05 gram of 99% benzoyl peroxide was dissolved in a mixture of 68.6 grams of n-butylmethacrylate, 1.4 grams of ethylene glycol dimethacrylate, and 30 grams of toluene. The solution above was mixed with an aqueous solution of 7.5 grams of polyvinylalcohol (MW 80,000, 80% hydrolyzed), 0.75 gram sodium oleate, and 300 grams of deionized water in a vessel provided with a homogenizer, a thermometer, a nitrogen inlet, and a reflux condenser. An initial stirring speed of 2500 rpm was employed for roughly 5 minutes with the introduction of nitrogen to form intermediately sized 4 - 12 micron beads. The stirring rate was reduced to 400 rpm to allow limited coalescence and form 10 - 14 micron beads with less than 25% variance in size distribution. Polymerization was carried out at about 75°C for 8 hrs (the temperature was actually increased slowly from room temperature to 75°C over 2 hrs. to avoid clumping). The beads were washed with water and acetone, and then dried at 80-100°C.

Example 2: Butylmethacrylate Beads, 5% Cross-linking

1.05 gram of 98% benzoyl peroxide was dissolved in a mixture of 66.5 grams of n-butylmethacrylate, 3.5 grams of ethylene glycol dimethacrylate, and 30 grams of toluene. The solution was mixed with an aqueous solution of 7.5 grams of polyvinylalcohol (MW 80,000, 88% hydrolyzed), 0.75 gram sodium oleate, and 300 grams of deionized water in a vessel provided with a homogenizer, a thermometer, a nitrogen inlet, and a reflux condenser. An initial stirring speed of 2500 rpm was employed with the introduction of nitrogen to form intermediately sized 4 - 12 micron beads. The stirring rate was reduced to 400 rpm to allow limited coalescence and form 10 - 14 micron beads with less than 25% variance in size distribution. The polymerization was carried out at about 75°C for 8 hrs. The product consisted of white beads between 10 and

14 microns in diameter. The beads were washed with water and acetone, and th n dried at 80-100°C.

Example 3: n-Hexylmethacrylate Beads

0.47 gram of 98% benzoyl peroxide was dissolved in a mixture of 30 grams of n-butylmethacrylate, 1.58 grams of ethylene glycol dimethacrylate, and 13.54 grams of toluene. The solution was mixed with an aqueous solution of 3.38 grams of polyvinylalcohol (MW 80,000, 88% hydrolyzed), 0.34 gram sodium oleate, and 135 grams of deionized water in a vessel provided with a homogenizer, a thermometer, a nitrogen inlet, and a reflux condenser. An initial stirring speed of 2500 rpm was employed with the introduction of nitrogen to form intermediately sized 4 - 12 micron beads. The stirring rate was reduced to 450 rpm to allow limited coalescence and form 10 - 14 micron beads with less than 25% variance in size distribution. The polymerization was carried out at about 75°C for 8 hrs. The product consisted of white beads between 10 and 14 microns in diameter. The beads were steam stripped to remove residual/monomers the beads and to maintain approximately 5% of the polyvinylalcohol on the beads' surface as a dispersing agent.

Example 4: Styrene-divinylbenzene beads

A monomer phase comprising 136 grams of styrene, 164 grams of divinylbenzene (55%), and 6 grams of benzoylperoxide was combined with 900 ml of an aqueous phase originally comprising 1250 ml of deionized water, 8.10 grams DOWFAX™ 2A1, 0.68 grams $K_2Cr_2O_7$ into a 3 liter glass reactor. (Two-thirds of the space in the reaction kettle was used to prevent overflow caused by foaming during the homogenization.) An oil bath was preheated to 75°C. The combined monomer in aqueous phases were homogenized at maximum speed until the desired particle size (2-4 microns) was obtained. This required 10 minutes. An external ice/water bath was not necessary to cool the solution during homogenization.

The reactor was then equipped with a condenser and a thermometer probe. A gas inlet adapter was attached to the

reaction vessel and the solution flushed with N_2 (set at 1-2 cfm). Agitation was begun at 400 rpm. When the solution reached approximated 65°C, agitation was reduced to 300 rpm and the solution stirred under the N_2 blanket.

The remaining 1/3 of aqueous solution (450 ml) was preheated by the oil bath to 70-74°C and added to the reaction vessel contents when the reaction temperature reached approximately 74°C. The agitation was then increased to 1000 rpm and then gradually reduced to 250 rpm. This process was completed in 10 minutes well before the solution temperature reached 75°C. An exotherm started slowly at 76°C. After the reaction reached 77°C, the temperature began to drop to 75°C. The reaction vessel contents were then stirred at 75°C for 8 hours.

After the reaction vessel had cooled, the contents were passed through a 400 mesh sieve and filter. The beads produced were filtered very easily, and were washed with 2 liters of deionized water twice with no acetone. The beads were then washed with 2 liters of acetone twice. The final beads were dried at 65°C overnight.

The beads were sized using a Malvern Instruments MASTER Particle Sizer. A small cell size was used with a dispersing agent of 100% filtered acetone and with a lens focal point of 63 millimeters. Table 4 presents the sizing results in volume percent with Table 5 presenting the results in number percent for the size of the particles.

The graph presented as Fig. 1 shows the number percent and volume percent from Tables 4 and 5, the "filled" histogram representing the number percent in each band, with the "empty" histogram representing the volume percent in each band.

Table 4 - Particle Size (Volume %)

Size microns	% under	% in band	Size microns	% under	% in band
118.4	100.00	0.0	11.1	100.0	0.4
102.1	100.0	0.0	9.6	99.6	0.6
88.1	100.0	0.0	8.3	99.0	1.5
76.0	100.0	0.0	7.2	97.5	3.3
65.6	100.0	0.0	6.2	94.1	5.0
56.6	100.0	0.0	5.3	89.1	7.7
48.8	100.0	0.0	4.6	81.3	13.7
42.1	100.0	0.0	4.0	67.7	23.1
36.3	100.0	0.0	3.4	44.6	21.7
31.3	100.0	0.0	3.0	22.9	10.2
27.0	100.0	0.0	2.6	12.7	2.1
23.3	100.0	0.0	2.2	10.5	1.2
20.1	100.0	0.0	1.9	9.3	0.6
17.4	100.0	0.0	1.6	8.7	0.8
15.0	100.0	0.0	1.4	7.9	1.1
12.9	100.0	0.0	1.2	6.8	

Table 5 (Particle Size (Number %)

Size microns	% under	% in band	Size microns	% under	% in band
118.4	100.00	0.0	11.1	100.0	0.0
102.1	100.0	0.0	9.6	100.0	0.0
88.1	100.0	0.0	8.3	100.0	0.0
76.0	100.0	0.0	7.2	100.0	0.0
65.6	100.0	0.0	6.2	100.0	0.0
56.6	100.0	0.0	5.3	99.9	0.3
48.8	100.0	0.0	4.6	99.6	0.5
42.1	100.0	0.0	4.0	99.1	1.3
36.3	100.0	0.0	3.4	97.8	2.2
31.3	100.0	0.0	3.0	95.6	1.7
27.0	100.0	0.0	2.6	93.9	0.6
23.3	100.0	0.0	2.2	93.3	0.4
20.1	100.0	0.0	1.9	92.9	0.2
17.4	100.0	0.0	1.6	92.7	0.9
15.0	100.0	0.0	1.4	91.8	1.4
12.9	100.0	0.0	1.2	90.4	

WHAT IS CLAIMED IS:

1. A process for preparing controlled characteristic beads comprising the steps of:

A) combining at least one monoethylenically unsaturated monomer with a polyethylenically unsaturated monomer and a monomer-soluble initiator to form a monomer phase;

B) combining the monomer phase with a liquid in which the monomer phase is substantially immiscible to form a reaction mixture such that when the monomer phase is vinyl in nature the liquid also includes a high molecular weight, high charge density surfactant, and when the monomer phase is acrylic in nature the liquid includes an organic polymer colloid suspending agent;

C) agitating the reaction mixture at an initial speed and temperature for a time sufficient to homogenize the reaction mixture, thereby producing first intermediate droplets, and subsequently reducing the agitation speed to a level sufficient to allow limited coalescence of the first intermediate droplets to form second intermediate droplets having size distribution with less than about 30% variance from the mean; and

D) polymerizing the second intermediate droplets to form solid, substantially non-porous beads.

2. A process in accordance with claim 1 wherein the monomer phase is vinyl in nature and includes said at least one monoethylenically unsaturated monomer selected from the group consisting of styrene, vinyltoluene, vinylxylene, vinyl chloride, vinylidene chloride, vinyl acetate, N-vinyl pyrrolidone, 2-vinyl pyridine, and vinyl alkyl ethers with alkyl ranging from C_2 - C_8, and said polyethylenically unsaturated monomer is selected from the group consisting of divinylbenzene, divinyltoluene, divinylxylene, divinylketone, divinylsulfide, alkyldivinylbenzenes having from 1 to 4 alkyl groups of 1 to 2 carbon atoms substituted on the benzene nucleus, alkyltrivinylbenzenes having 1 to 3 alkyl groups of 1

to 2 carbon atoms substituted on the benzen nucleus, trivinylnaphthalenes, and polyvinylanthracenes.

3. A process in accordance with claim 1 wherein the monomer phase is acrylic in nature and said at least one monoethylenically unsaturated monomer is selected from the group consisting of acrylic acid, methacrylic acid, alkyl acrylates having alkyl group of C_1 - C_6, normal and branched alkyl methacrylates having alkyl group of C_1 - C_6, and acrylamide, and said polyethylenically unsaturated monomer is a glycol diacrylate selected from the group consisting of

$$CH_2=C(R^1)C(O)O[(CH_2)_yCH(R^2)O]_zC(O)CH(R^1)C=CH_2$$

wherein R^1 is H or CH_3;
R^2 is H or CH_3;
y is 1 to 5; and
z is 1 to 14.

4. A process in accordance with claim 3 wherein R^1 is CH_3, R^2 is CH_3; y is 1, and z is 1.

5. A process in accordance with claim 1 wherein said beads have diameter ranging from about 0.1 to 200 microns.

6. A process in accordance with claim 1 wherein said beads have diameter ranging from about 2 to 20 microns.

7. A process in accordance with claim 1 wherein said beads have diameter ranging from about 6 to 9 microns.

8. A process in accordance with claim 1 wherein the monoethylenically and polyethylenically unsaturated monomers are provided in percentages such that substantially all the product beads have specific gravity ranging from about 0.9 to about 1.2.

9. A process in accordance with claim 1 wherein the monoethylenically and polyethylenically unsaturated monomers are provided in percentages such that substantially all the

product beads have specific gravity ranging from about 1.0 to about 1.045.

10. A process in accordance with claim 1 wherein the monoethylenically and polyethylenically unsaturated monomers are provided in percentages such that substantially all the product beads have specific gravity ranging from about 1.045 to about 1.056.

11. A process in accordance with claim 1 wherein the monoethylenically and polyethylenically unsaturated monomers are provided in percentages such that substantially all the product beads have specific gravity ranging from about 1.056 to about 1.07.

12. A process in accordance with claim 1 wherein the high molecular weight, high charge density surfactant is selected from the group consisting of anionic, alkylated, disulfonated diphenyloxides having molecular weight of at least about 400, and where the hydrophobic alkyl group is normal or branched, having from about 5 to about 16 carbon atoms.

13. A process in accordance with claim 1 wherein the organic polymer colloid suspending agent is selected from the group consisting of organic polymer polyols having molecular weight of at least 50,000, and degree of hydrolysis of at least 50%.

14. A process for preparing controlled characteristic beads comprising the steps of

forming a monomer phase comprising styrene, divinylbenzene, and an initiator, at least a known percentage of the styrene substituted with vinyltoluene;

combining said monomer phase with a solution that is substantially immiscible with the monomer phase, the solution including a high molecular weight, high charge density surfactant, to form a reaction mixture;

agitating the reaction mixture for a speed and time sufficient to homogenize th reaction mixture and form first intermediate size monomer droplets;

reducing agitation to allow limited coalescence of the first intermediate size monomer droplets to form second intermediate size monomer droplets having size distribution variance of at most 30%; and

polymerizing the second intermediate size monomer droplets to form substantially solid, non-porous product beads of styrene-vinyltoluene copolymer crosslinked with divinylbenzene having a known percentage of cross-linking.

15. Process in accordance with claim 14 wherein said product beads have size ranging from about 0.1 to about 200 microns.

16. Process in accordance with claim 14 wherein said product beads have size ranging from about 2 to about 20 microns.

17. Process in accordance with claim 14 wherein said product beads have size ranging from about 6 to about 9 microns.

18. Method of using controlled characteristic beads as a separation aid in centrifugation comprising combining a solution including at least one narrow density range of beads, the beads having size distribution of at most about 30%, with a sample having components to be separated to form a test sample, and centrifuging the resulting test sample for a time and rate sufficient to separate the components in the test sample into at least two substantially distinct bands of components, the beads thereby functioning to enhance visualization of the separated components.

19. Method in accordance with claim 18 wherein said at least two narrow density ranges comprises a first standard having a predetermined specific gravity, a second standard having a predetermined specific gravity and a third standard having a predetermined specific gravity, wherein the specific gravity of the first standard is less than the specific gravity of the second standard, and the second standard specific gravity is less than the specific gravity of the third standard.

20. Method in accordance with claim 19 wherein the specific gravity of the first standard ranges from about 1.04 to about 1.05, the specific gravity of the second standard ranges from about 1.05 to about 1.06, and the specific gravity of the third standard ranges from about 1.06 to about 1.08.

21. Method in accordance with claim 20 wherein the specific gravity of the first standard ranges from about 1.043 to about 1.047, the specific gravity of the second standard ranges from about 1.054 to about 1.058, and the specific gravity of the third standard ranges from about 1.065 to about 1.075.

22. Method in accordance with claim 19 wherein the specific gravity of the first standard approximates the specific gravity of blood platelets, the specific gravity of the second standard approximates that of lymphocytes and monocytes, and the specific gravity of the third standard approximates that of granulocytes.

23. Method in accordance with claim 19 wherein the beads in each standard density range have size ranging from about 0.1 microns to about 200 microns.

24. Method in accordance with claim 23 wherein the beads in each standard density range have size ranging from about 2 microns to about 20 microns.

25. Method in accordance with claim 24 wherein the beads in each standard density range have size ranging from about 6 microns to about 9 micr ns.

26. Method in accordance with claim 19 wherein the second standard beads have their surface modified with a moiety selected from the group consisting of SO_3^- and CO_2^- to facilitate adsorption of dyes.

27. A method of using controlled characteristic beads to calibrate a hematology centrifuge comprising combining at least two narrow specific gravity ranges of beads with blood to form a control, the blood having substantially all platelets, lymphocytes/monocytes, and granulocytes removed therefrom, the beads in each specific gravity range having a size distribution of at most 30% variance, and centrifuging the control at a speed and for a period of time to determine if the centrifuge is calibrated.

28. Method in accordance with claim 27 wherein said at least two narrow specific gravity ranges comprises a first standard having a specific gravity, a second standard having a specific gravity, and a third standard having a specific gravity, wherein the specific gravity of the first standard is less than the specific gravity of the second standard, and the specific gravity of the second standard is less than the specific gravity of the third standard.

29. Method in accordance with claim 28 wherein the specific gravity of the first standard ranges from about 1.04 to about 1.05, the specific gravity of the second standard ranges from about 1.05 to about 1.06, and the specific gravity of the third standard ranges from about 1.06 to about 1.08.

30. Method in accordance with claim 29 wherein the specific gravity of the first standard ranges from about 1.043 to about 1.047, the specific gravity of the second standard rang s from ab ut 1.054 t about 1.058, and the specific

gravity of the third standard ranges from about 1.065 to about 1.075.

31. Method in accordance with claim 29 wherein the specific gravity of the first standard approximates the specific gravity of blood platelets, the specific gravity of the second standard approximates that of lymphocytes and monocytes, and the specific gravity of the third standard approximates that of granulocytes.

32. Method in accordance with claim 29 wherein the beads in each standard have size ranging from about 2 microns to about 20 microns.

33. Method in accordance with claim 29 wherein the beads in each standard have particle size ranging from about 6 microns to about 9 microns.

34. Method in accordance with claim 29 wherein the second standard beads have their surface modified with a moiety selected from the group consisting of SO_3^- and CO_2^- to facilitate adsorption of dyes.

35. A kit for calibrating centrifuges, which kit comprises

a centrifugable container adapted to having disposed therein blood having substantially all platelets, lymphocytes and monocytes, and granulocytes removed therefrom, adapted to be replaced respectively with three sets of solid, non-porous beads contained in separate containers;

the first set of beads having specific gravity approximately of platelets, the second set having specific gravity approximating lymphocytes and monocytes, and the third set having specific gravity approximating granulocytes;

the beads in each specific gravity range having size distribution variance of at most 30%;

the beads comprising c polym rs f at least one monoethylenically unsaturated monomer selected from th group consisting of acrylic acid, methacrylic acid, alkyl acrylates having alkyl group of C_1 - C_6, normal and branched alkyl methacrylates having alkyl group of C_1 - C_6, and acrylamide, and a glycol diacrylate polyethylenically unsaturated monomer selected from the group consisting of

$$CH_2=C(R^1)C(O)O[(CH_2)_yCH(R^2)O]_zC(O)CH(R^1)C=CH_2$$

wherein R^1 is H or CH_3;
R^2 is H or CH_3;
y is 1 to 5; and
z is 1 to 14.

36. The kit of claim 35 wherein one or more sets of beads is surface modified with chemical moieties selected from the group consisting of SO_3^- and CO_2^-, the moieties in turn coupled with dyes.

37. The kit of claim 36 wherein the centrifugable container is sealed and contains a standard modified blood product having the platelets, lymphocytes and monocytes, and granulocytes replaced substantially with the three sets of differing specific gravity beads.

38. A kit for enhancing visibility of centrifugal separation between one or more components of a sample, which kit comprises

at least two different density bead groups in the same or different containers, the beads being solid and substantially non-porous having size ranging from about 2 microns to about 20 microns, and further having size distribution of no more than about 30% variance from the mean bead diameter, the beads adapted to be added to a sample to be centrifuged,

the beads comprising copolymers of at least one monoethylenically unsaturated monomer selected from the gr up consisting of acrylic acid, methacrylic acid, alkyl acrylates having alkyl gr up f C_1 - C_6, normal and

branched alkyl methacrylates having alkyl group of C_1 - C_6, and acrylamide, and a glycol diacrylat polyethylenically unsaturated monomer s lect d from the group consisting of

$$CH_2=C(R^1)C(O)O[(CH_2)_yCH(R^2)O]_zC(O)CH(R^1)C=CH_2$$

wherein R^1 is H or CH_3;
R^2 is H or CH_3;
y is 1 to 5; and
z is 1 to 14.

39. The kit of claim 38 wherein the beads of at least one group are surface modified with moieties selected from the group selected from the group consisting of SO_3^- and CO_2^- to adsorb dyes from a sample.

40. The kit of claim 38 wherein the sample is human whole blood and the at least two different density groups comprises a first set of beads having specific gravity ranging from about 1.04 to about 1.05, a second set of beads having specific gravity ranging from about 1.05 to about 1.06, and a third set of beads having specific gravity ranging from about 1.06 to about 1.08.

41. The kit of claim 39 wherein the sample is human whole blood and the at least two different density groups comprises a first set of beads having specific gravity ranging from about 1.04 to about 1.05, a second set of beads having specific gravity ranging from about 1.05 to about 1.06, and a third set of beads having specific gravity ranging from about 1.06 to about 1.08.

42. Method of using narrow size distribution beads as opacifiers for biaxially oriented plastic compositions comprising

providing a resin combination comprising a thermoplastic polymer matrix having dispersed therein as distinct phases a plurality of substantially spherical, solid, non-porous beads having size distribution variance of at most 30%;

the beads made from monomers selected from the group consisting of at least one monoethylenically unsaturated monomer selected from the group consisting of styrene, vinyltoluene, vinylxylene, vinyl chloride, vinylidene chloride, vinyl acetate, N-vinyl pyrrolidone, 2-vinyl pyridine, and vinyl alkyl ethers with alkyl ranging from C_2 - C_8, and a polyethylenically unsaturated monomer selected from the group consisting of divinylbenzene, divinyltoluene, and divinylxylene;

forming an unoriented film of said resin combination; and

biaxially orienting said film to an extent sufficient to opacify the same.

43. Method in accordance with claim 42 wherein the beads have size ranging from about 0.1 micron to about 10 microns.

44. Method in accordance with claim 43 wherein the matrix is polypropylene.

45. The product produced by the method of claim 1.

46. The product produced by the method of claim 2.

47. The product produced by the method of claim 3.

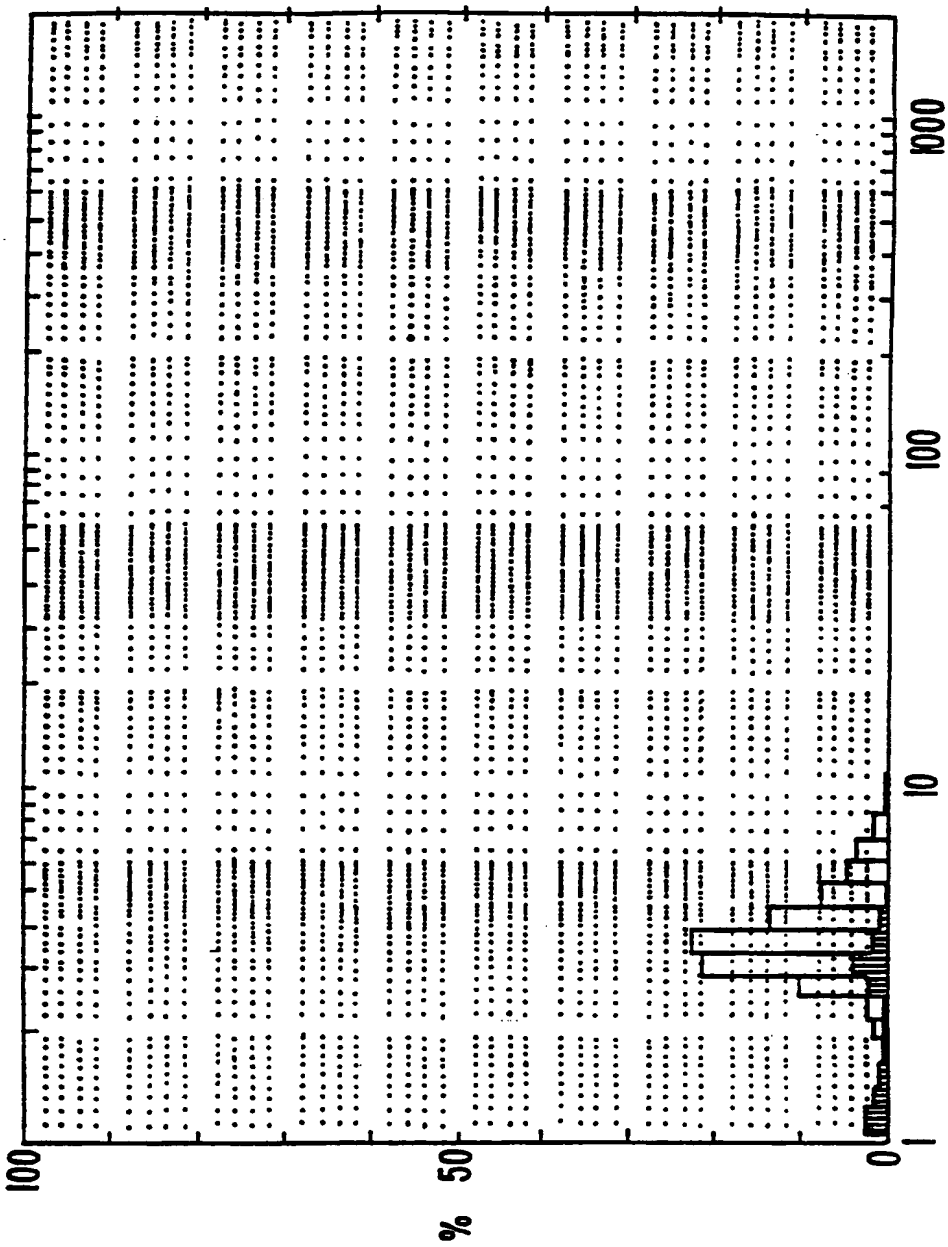

FIG. 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03212

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :B01D 33/15; C08F 2/00
US CL :210/782; 526/88
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/782; 526/88

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 2,932,629 (WILEY) 12 April 1960, column 3, line 11 to column 5, line 61; column 9, line 60 to column 10, line 17; examples 1 and 2.	1-17 and 45-47
A	"The Scandinavian Journal of Clinical & Laboratory Investigation", vol. 21, Supplemental 97, (BOYUM), pages 9-29, "Separation of Leukocytes From Blood and Bone Marrow".	18-26
A	"Separation of Blood Leukocytes, Granulocytes and Lymphocytes", Tissue Antigens, (BOYUM), vol. 4, pages 269-274., (15 January 1974).	18-26
A	"A Method for the Recognition and Separation of Human Blood Monocytes on Density Gradients", Blood, November 1976, (LODS ET AL.), vol. 48, No.5, pp 731-742.	18-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 JULY 1992	14 AUG 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer Joseph L. Schofer Telephone No. (703) 300-2351

Form PCT/ISA/210 (second sheet)(July 1992)★

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

GROUP I, Claims 1-26, and 45-47 drawn to a process for making, product, and process for using controlled characteristic beads, classified in class 526, subclass 88.

GROUP II, Claims 27-34 drawn to a method of using beads to calibrate a hematology centrifuge, classified in class 210, subclass 646.

GROUP III, Claims 35-41 drawn to a kit for calibrating centrifuges, classified in class 206, subclass 540.

GROUP IV, Claims 42-44 drawn to a method of using beads as opacifiers for biaxially oriented plastic compositions, classified in class 428, subclass 402.